Hyaluronan Concentration and Size Distribution in Human Knee Synovial Fluid: Variations with Age and Cartilage Degeneration

INTRODUCTION: The human knee commonly deteriorates with aging, with two of the earliest changes being a structural roughening of the superficial zone of the articular cartilage in association with a functional decrease in tensile stiffness and strength.1,2 With osteoarthritis (OA), further deterioration of the superficial zone occurs, with structural erosion and further mechanical weakening.3 One possible mechanism for these changes is age- and disease-related deterioration of the lubricant quality of knee synovial fluid. Hyaluronan (HA) is one of the primary lubricants of synovial fluid (SF).4 The friction-lowering properties of HA are dependent on its molecular mass (M), with lubrication properties being better for higher-M, forms of HA. In OA compared to normal knees, the HA concentration appears decreased,4,6 with a shift to lower M, forms of HA.6,7 However, the characteristics of HA in aging without OA are unknown. The hypothesis tested in this study was that the HA concentration and MW distribution in human knee SF fluid varies with adult age and cartilage degeneration. The aims were to determine the overall concentrations of protein and HA, as well as HA M, distribution, in human knee SF (hSF) and their relationships with age and grade of joint degeneration.

METHODS: Synovial Fluids. hSF was aspirated from one or both knee joints (n=66) of cadaveric donors (n=39, age mean±SD of 64±19 yr and range of 23-91 yr) that were collected from tissue banks with 72% of death (range 24-72, 50±17). Donors were excluded if they had a history of knee arthritis or joint trauma or if osteophytes were present. An overall joint grade was determined for the knee articular cartilage as the sum of modified Outerbridge macroscopic grades of nine regions per condyle, three regions per trochlear region, nine regions for each tibial plateau and nine regions for the patella as described by the ICRS.9,10 Scores could range from 48-192 for completely normal-degenerate knees, and a maximum score of 121 was considered for inclusion in order to exclude OA knees.

Biochemical Analysis of Putative Boundary Lubricants. Portions of hSF samples were assayed for the concentrations of total protein and HA, as well as the M, distribution of HA ([protein], [HA], [HA(low M, high M)]). HA was quantified with an ELISA-like assay.11 The concentration of HA in M, ranges of 0.13-0.25, 0.25-0.5, 1.25, 1.25-2.5, and 2.5-7 MDa was determined by protease-K digestion of a portion of each sample and assessing the S. Hyaluronidase-sensitive portion on electrophoresis in 1% agarose gel with HA standards of varying.12

Statistics. The quantities, joint grade, [protein], [HA], [HA(0.13-0.25 MDa)], [HA(0.25-0.5 MDa)], [HA(0.5-1.25 MDa)], [HA(1.25-2.5 MDa)], and [HA(2.5-7 MDa)] were tested for relationships (1) between left and right knees with comparison of slopes to 1 by t-test, as well as (2) with age and (3) cumulative joint grade by univariate linear regression.

RESULTS: Left and right knees from individuals showed a number of similarities. Cumulative joint grades (not shown, R²=0.99, p<0.01 vs a slope of 1), [HA] (Fig. 1A), [protein] (Fig. 1B), [HA(1.25 MDa)], and [HA(2.5-7 MDa)] that were strongly correlated. Thus, for subsequent analyses, left and right knee characteristics from an individual were averaged.

With age, [HA] decreased significantly (Fig. 2A), whereas [protein] did not vary detectably (Fig. 2B). The rate of decrease in [HA] with age was −7.8% / decade. In particular, high M, forms of HA, [HA(1.25 MDa)] and [HA(2.5-7 MDa)] tended to decrease with age at a rate of −6.8% / decade and −8.2% / decade, respectively (Fig. 2C,D).

None of the quantities analyzed varied significantly with cumulative joint grade (Fig. 3A,B).

DISCUSSION: The [HA] and [protein] values agree reasonably with the data available in the literature, suggesting that the post-mortem sampling method provides an appropriate indicator of SF status. The similarities in SF properties between left and right knees from an individual are striking, and may reflect subject-specific physiological processes. The decrease in [HA] with age in the absence of clinical osteoarthritis suggests this change may be an important factor in the age-related deterioration of knee articular cartilage.

SIGNIFICANCE: In non-OA knees, the concentration of hyaluronan decreases with age by −7.8% / decade, and this decrease may contribute to diminished lubrication and cartilage degeneration.

ACKNOWLEDGEMENTS: NIH AG007996.